

INFLUENCE OF REACTION ON INTER-RELATIONS BETWEEN THE PLANT AND ITS CULTURE MEDIUM

BY

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I. INFLUENCE OF THE REACTION OF THE MEDIUM UPON THE PLANT

INTRODUCTION*

The reaction of the substrate in which the roots of plants develop is of obvious importance to the life of the plants. Earlier plant physiologists have neglected this factor, and it was not until recently as a result of studies on the intensity of the acidity of the soil solution of certain soils, on the one hand, and the relative resistance of different varieties of plants to alkaline conditions in certain types of 'alkali soils,' on the other, that the significance of this factor was fully realized.

Since the preliminary studies of Pantanelli²⁶ and Hoagland,¹⁵ several investigators have attacked the problem. The practical, as well as theoretical importance of a more thorough understanding of the influence of the reaction of the culture medium on the growth and metabolism of plants seemed to warrant the investigation here described.

The object was twofold: (1) a study of the effect of various concentrations of hydrogen ions on the external appearance and growth of the more common agricultural plants; (2) the effect of the reaction on the metabolism of these plants.

* The writer wishes to acknowledge his indebtedness to Professor D. R. Hoagland for advice and kindly suggestions during the course of the investigation.

For obvious reasons, it was impossible to employ more than a few types of plants to accomplish these aims; hence plants were selected which were adapted to the methods of experimentation, and which may be considered as representative of the majority of field crops. These were alfalfa (*Medicago sativa*), cotton (*Gossypium herbaceum*, Durango variety), cucumbers (*Cucumis sativa*, White Spine variety), Bermuda grass (*Cyanodon dactylon*), corn (*Zea mais*, White Dent field corn), barley (*Hordeum vulgare*, Beldi variety), and peas (*Pisum sativum*, Canada field), the latter two being the principal ones used in the study of the inter-relations between the metabolism of the plant and the reaction of the culture solution.

Owing to the complexity of the soil and the reactions taking place therein and because of the many complicating factors which enter when sand cultures are used, solution cultures were employed exclusively.

EXPERIMENTAL

Baker's analyzed salts and the ordinary distilled water of the laboratory were used in making all culture and stock solutions. The stock solutions were those used regularly in this laboratory. Table 1 gives the weights of salts added to 18 liters of water to make up those solutions.

TABLE 1
WEIGHTS OF SALTS DISSOLVED IN 18 LITERS OF THE DISTILLED WATER TO
MAKE UP THE STOCK SOLUTIONS

Solution I	Solution II	Solution III
KNO ₃ : 1200 grams MgSO ₄ : 679 grams	Ca(NO ₃) ₂ : 1805 grams	KH ₂ PO ₄ : 900 grams

In table 2 is given the composition of the culture solution used throughout in this investigation (except where otherwise stated). This solution was made by adding 80 c.c. of solution I, 40 c.c. of solution II, 480 c.c. of solution III, and 24 grams of NaNO₃ to 44 liters of water.

TABLE 2
COMPOSITION OF CULTURE SOLUTION EXPRESSED AS EQUIVALENTS, PER LITER

K	NO ₃	H ₂ PO ₄	Ca	Mg	SO ₄	Na	PH
0052	.0087	.0040	.0011	.0007	.0007	.0064	4.9

Iron was supplied in the form of ferrie tartrate, one cubic centimeter of a 0.5 per cent solution being used per liter of culture solution.

In figure 1, the titration curve of the solution is reproduced. This was obtained colorimetrically. By interpolation the amounts of acid or alkali to be added to eleven liters of the solution to obtain any desired P_H within the useful range can be found from this graph.

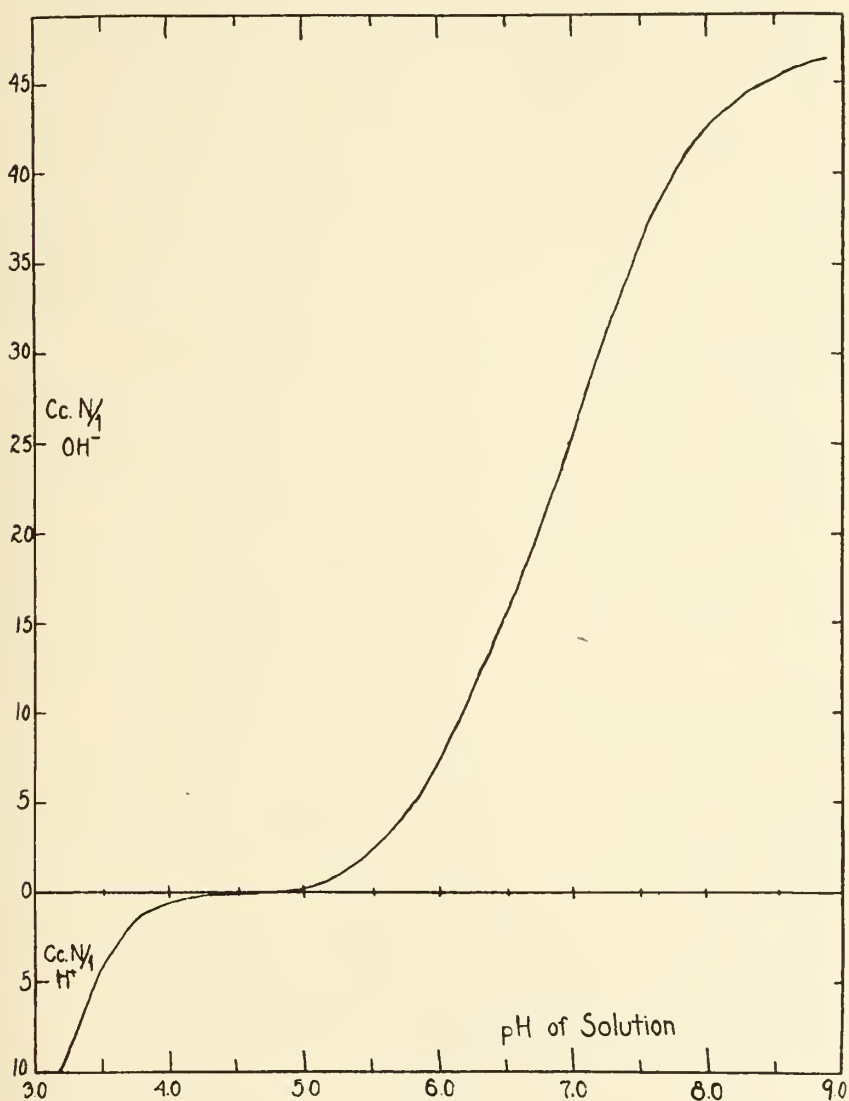


Fig. 1

In an effort to keep the composition of the solution as constant as possible over the entire range of reactions used, the concentrations of Ca and Mg were kept low, and were regulated by the amount of

calcium which will remain in solution at P_H 8.0. A precipitate usually occurred at P_H 8.5 and often at 8.0 after a few days. A comparatively high concentration of phosphate was used, on the other hand, in order to increase the buffer effect of the solution. Unfortunately, the buffer effect varies over different ranges of reactions.

This serious defect may be partly remedied by the addition of an acid with a dissociation constant of about 4.5 and a base with a constant of about 5.5. The only non-toxic acids having the desired constant are organic acids, e.g., citric acid. Owing to the danger of excessive bacterial growth in solutions containing organic matter, however, these non-toxic, organic acids cannot be used satisfactorily (Salter and McIlvaine²⁷). Ammonium hydroxide may be used to supplement the buffer effect of the phosphate at P_H 8 to P_H 10, but the advantages to be gained here are small and the presence of the ammonium ion may introduce complicating factors.

Growth in the culture solution was very satisfactory if changes were made weekly. Sulfuric acid and sodium hydroxide were used to regulate the P_H values of the solutions. Measurements of the reaction were made by the indicator method of Clark and Lubs.¹³ Frequent use was also made of a Hildebrand-type hydrogen electrode.

The plants were germinated between sheets of wet paper toweling and the usual methods of solution culture technique followed. At first properly covered Mason jars of 950 c.c. capacity were used as containers. In each jar, three plants were grown, ten jars being employed for each P_H tested.

The plants were grown in series of solutions having the following initial values: 4.0, 4.5, 5.0, 6.0, 7.0, 8.0, 8.5, and 9.0. Since the reaction changed very rapidly in the direction of neutrality, the solutions were renewed every second day. These frequent renewals, however, did not prevent the reactions of the solutions from being changed considerably during the later stages of growth. The maximum changes in reactions are tabulated in table 3. The plants were grown from 3 to 4 weeks, within which time sufficient growth was made to determine at which reactions they were affected adversely.

TABLE 3
MAXIMUM AND MINIMUM VALUES OF THE REACTIONS AT TIME OF CHANGE

Initial P_H of Series	4.0	4.5	5.0	6.0	7.0	8.0	8.5
Maximum and minimum P_H at time of change	4.1-4.4	4.6-5.0	5.0-5.2	6.0-6.1	7.0-6.9	8.0-7.9	8.4-8.0

We assume naturally that the slightly increased concentrations of Na ions and SO_4 ions used in regulating the P_H values of the different solutions have no effect on the plant, and that all differences in the external characteristics are caused directly or indirectly by the activities of the hydrogen or hydroxyl ions. The influence of the different reactions was determined by the relative weights of plants grown in the different solutions, the length and appearance of the roots, and the height and color of the tops.

The general effect of excessive acidity is very characteristic, and is the same for all the plants used in the experiment. If the culture solution is injuriously acid, the roots thicken and soon become a dull white in color which is easily distinguishable from the silky white appearance of normal roots. Depending upon the degree of acidity, the roots may stop growing in length entirely or may grow only slowly. In the latter case, they become knobby, because of the excessive development of laterals which penetrate the outer layers of the root with apparent difficulty. Lateral roots may develop to within a few millimeters from the growing tip. If the injury is not too severe the roots recover very rapidly when placed in a more favorable solution. The tops of the plants show a marked stimulation in growth and general vigor, as a rule, when compared with the plants grown in a more favorable solution. The stimulation, however, is of short duration and after two weeks they begin to lag behind. Similar results were obtained by Hixon.¹⁴

An injurious alkalinity of the culture solution is very readily recognized by a yellowish discoloration of the roots. In extreme cases, the roots become gelatinous and soon disintegrate. At first the tops showed no differences in size and vigor as a result of injury to the roots when compared with the tops of plants growing in a more favorable solution. After two to three weeks, however, a decided stunting was noticeable, and chlorosis of the new leaves set in.

Chlorosis is generally ascribed to the lack of available iron. This was probably the main cause of the chlorosis of those plants grown in the alkaline series. That excessive concentrations of hydroxyl ions, however, may cause chlorosis directly seems certain from the following considerations.

A distinct test for iron could be demonstrated in a solution kept at P_H 8.5 even after chlorotic plants had been growing in it for a week. Cucumbers and alfalfa will show chlorosis at P_H 7 within two weeks; at this reaction neither barley nor peas show any chlorosis

even after nearly two months' growth. By this time, one would expect the supply of iron stored in the seeds of the latter plants to be depleted. Gile and Carrero⁸ found that ferric tartrate supplied the necessary iron to plants grown in solutions which they thought to be alkaline.

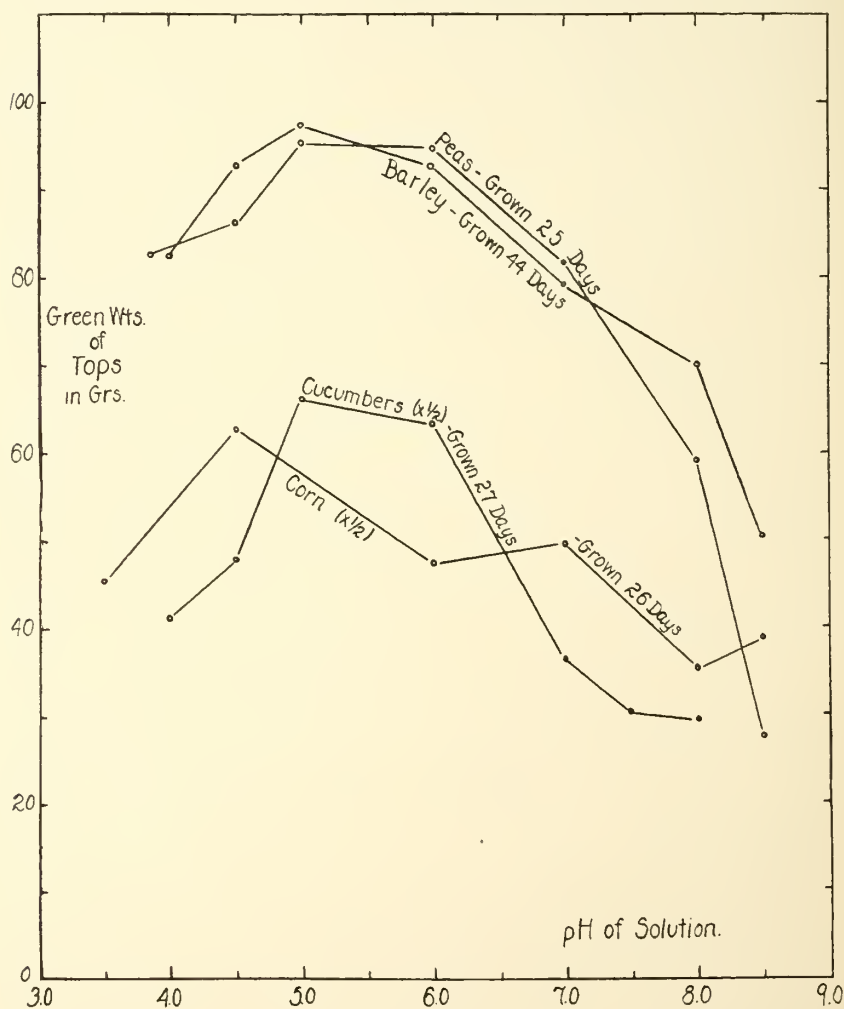


Fig. 2

It may be objected that the iron is not translocated from the roots to the tops in the case of the plants growing in an alkaline solution, and hence the plants are nevertheless suffering from a lack of iron. The reaction of the root juices, expressed after freezing, indicates,

however, that it is hardly possible for the increased concentration of the hydroxyl ions to interfere with the translocation.

All the plants grown on the acid side of P_H 6.0 were deep green; above this reaction the color gradually became paler green, merging into complete chlorosis at the higher P_H values.

It is apparent that the plant is influenced strongly by the reserve store of food material in the seed. Great care must be taken in making any deductions from the experiments in which the plants have been grown for a short period of time only.

A much more thorough study of the problem has been made using the technique described below. The results of the experiments just discussed are therefore summarized in table 4 without further detail here.

TABLE 4
EFFECT OF ACIDITY AND ALKALINITY ON GROWTH OF VARIOUS PLANTS

Plant	P_H injuriously acid	P_H at which optimum growth takes place	P_H injuriously alkaline	Remarks
Alfalfa	4.2-4.5	4.8-6.0	7.0	Very sensitive
Cotton	4.2-4.5	5.0-7.0	8.0	Fairly resistant
Cucumbers	4.2-4.5	4.8-6.0	7.0	Very sensitive
Barley	4.2-4.5	4.5-7.0	8.0	Resistant
Bermuda grass	4.2	4.5-8.0	9.0	Highly resistant

All the varieties of plants tested, except the Bermuda grass, were affected adversely by approximately the same intensity of acidity. Alfalfa and cucumbers were affected much more severely, a fact which is correlated with their greater sensitiveness to alkaline conditions. In all cases, the best growth was made when the reaction of the culture solution was between P_H 5 and P_H 6. It may be of interest here to note that Fred and Davenport⁶ found the critical point for the growth of alfalfa bacteria to be at P_H 4.9. This reaction is well within any possible critical range for the host plant.

The use of the technique described above involves an excessive amount of labor and errors are unavoidable. At best, we are unable to control the reactions of the solutions satisfactorily. The advantages of the technique evolved later and described below will at once be evident.

Whereas with the former technique, 30 plants were grown in ten different jars at every P_H value in the experiment, all 30 plants were now grown in one three-gallon (eleven liter) earthenware crock. The

plants were supported as before in perforations on a cork sheet made by binding together three 12" x 4" x $\frac{3}{8}$ " cork slabs with two strips of wood nailed on the edges. To prevent lateral movements, small pieces of wood were nailed on the underside of the overlapping corners. Slabs of wood $\frac{7}{8}$ " thick serve the purpose even better, since the plants can be supported more firmly in them. These slabs must be soaked thoroughly in hot pure paraffin so as to prevent the absorption of water. By growing all thirty plants in this large volume of solution, the effect of the inherent variability of the plants is minimized and most of the experimental errors are eliminated. The roots can

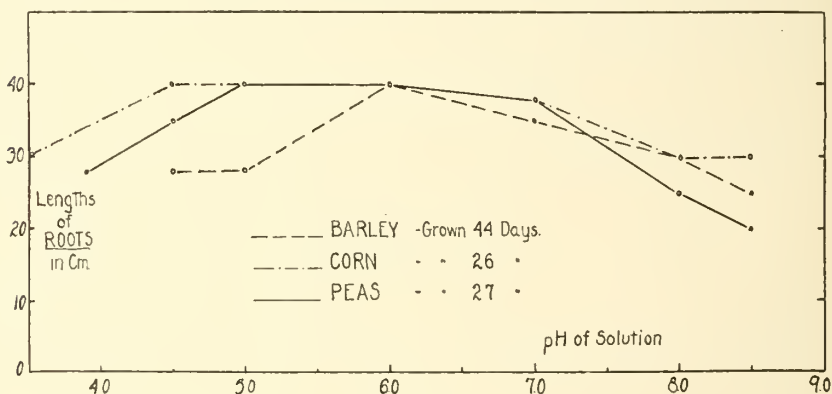


Fig. 3

be inspected readily and the P_H of the solution can be adjusted conveniently, rapidly, and as frequently as desired. The P_H is adjusted by withdrawing 5 c.c. samples of the solution and determining the reaction colorimetrically. The amounts of acid or alkali which must be added to bring the P_H to the original value are read off from figure 1, and the required quantities added to the solution. Figure 1 applies strictly to only the fresh solution. Within a week, however, the composition of the solution did not change sufficiently to invalidate the method. The solutions were changed every week and the P_H adjusted twice a day, i.e., in the morning and evening. During the later stages of growth, this becomes necessary more frequently in the case of plants growing at the reactions P_H 4.0-5.0 and P_H 8.0-9.0. Over these ranges, the buffer effect of the solution is relatively small and the power of the plant to change the P_H of the solution is increased (see figs. 4 and 5). All the plants experimented with showed a tendency to change the P_H of the solutions to a value between P_H 6.2 and 6.8.

Because of the lack of time, it was not possible to subject all the plants used in the former experiments to these better controlled methods. This was done, however, with four widely different types of plants, namely, barley, peas, cucumbers, and corn.

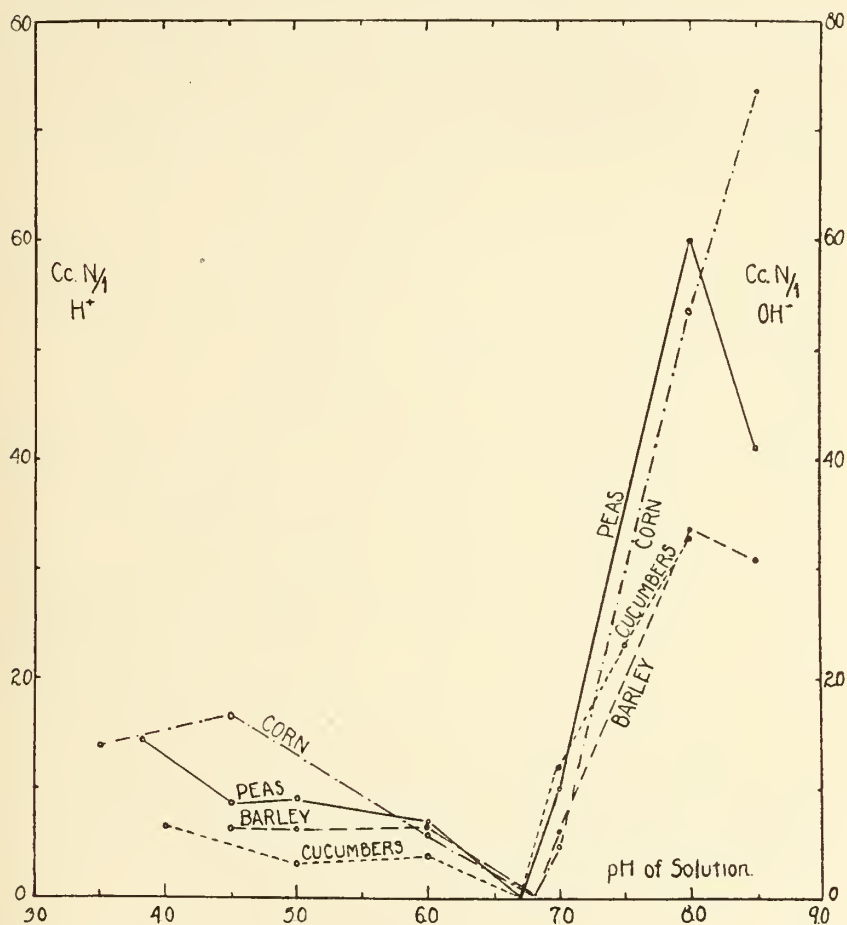


Fig. 4

In table 5, one experiment with peas is summarized. In the first column are given the desired reactions of the solution, in the second the highest and lowest P_H values reached during the course of the experiment, and in the seventh the number of cubic centimeters of normal hydrogen (sulphuric acid), or normal hydroxide ions (sodium hydroxide), added during the entire period of growth to replace that neutralized by the plants.

In figure 2, the green weights of the tops of 30 plants of the four types are plotted against the P_H of each series (see column 1, table 5), and in figure 3, the length of the roots. Since neither the change of P_H nor the change in the total molality of acid or base with time is an arithmetic function, it is impossible to calculate an average P_H . The true average P_H values differ only by a small amount from the desired P_H such that the given curves are not greatly different from the curves which would be obtained if the true average reactions were used. The differences are within the limits of the experimental error.

TABLE 5
SUMMARY OF A TYPICAL EXPERIMENT WITH PEAS

Desired P_H of series	Maxi- mum range of P_H	Days grown	No. of plants	Green weight of tops gms.	Length of roots cms.	C.C. N/1 Reagent neutralized		Remarks
						Acid	Alkali	
3.9	3.9-4.0	25	30	82.8	28	14.8	Roots severely injured
4.5	4.5-4.7	25	30	86.2	35	8.8	Very slight injury to roots
5.0	5.0-5.2	25	30	95.3	40	8.9	Best growth
6.0	6.0-6.1	25	30	94.2	40	7.0	Best growth
7.0	7.0-6.9	25	30	81.1	38	.	8.0	
8.0	8.0-7.9	25	30	59.4	25	.	60.0	Tops slightly chlorotic
8.5	8.5-8.3	25	30	27.6	20	.	41.0	Roots badly injured. Tops chlorotic

The juices of the plants were needed for other experiments, so the dry weights were not determined. For the present purpose, the green weights of the tops give a reliable criterion of the general vigor and size of the plants. The differences in the weights of the barley and pea plants can hardly be considered as significant in themselves on account of the inherent variability of the plants. If the observations on the other effects are taken into consideration, however, it becomes evident that the small differences in weight are true expressions of the effect of the corresponding reactions on the growth of the plants.

The maximum changes in P_H brought about by all four types were the same as that given for peas in column 2, table 5, except in the case of corn, grown in the alkaline solution, where the reaction frequently reached the P_H 8.1. Since four widely different types of plants were used, the curves may be considered as a definite measure of the effect of the reaction of the culture medium on the growth of

most agricultural plants as indicated by the yield. They show unmistakably that the optimum range of the reaction for the propagation of these plants in solution cultures is between P_H 4.5 and 6.0, and agree substantially with the results found with the earlier method of experimentation (see table 4). In figure 4, the amounts of normal acid or alkali neutralized by 30 plants during the first 25 days of

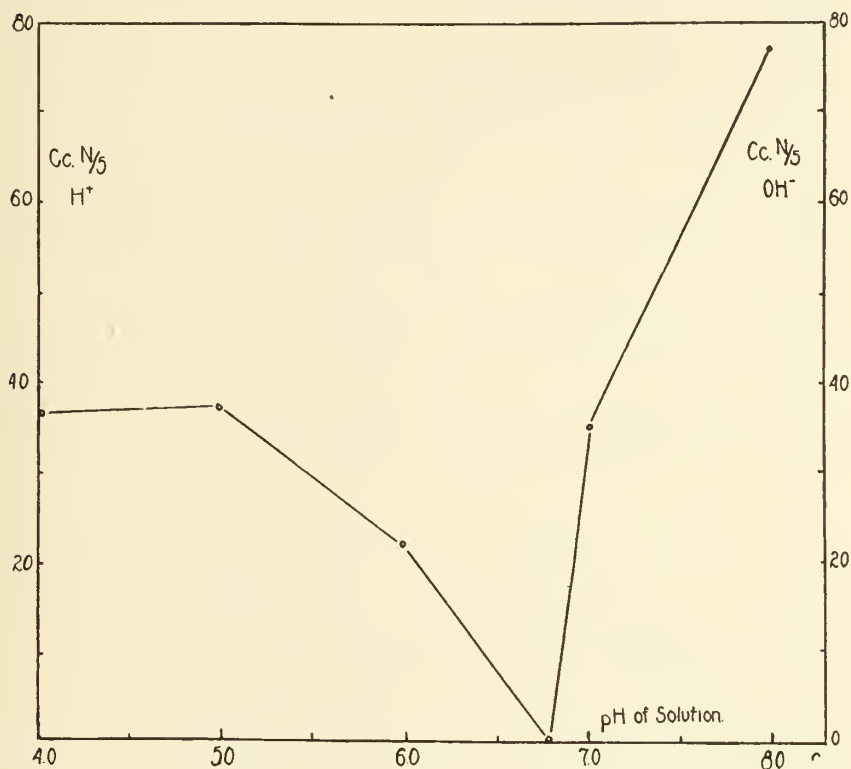


Fig. 5

growth is represented graphically for each type. These curves are not strictly comparable, since the plants were grown at different times of the year. The amounts of acid or alkali neutralized within a definite period of time depend largely upon the rapidity of growth.

Although the curves are only of a qualitative significance, they are very expressive of the power of the plant to overcome any unfavorable acidity or alkalinity, especially the latter. This power is of obvious importance to the plant and must form an integral part of any study of acid or alkali resistant crops, either in the soil or in

solution culture. Under natural conditions, the plant has to contend with the reaction of the medium in which its roots are immersed or imbedded from the time of germination to maturity. If the medium is sufficiently highly buffered or is continually renewed, such that little or no change of reaction is brought about under the influence

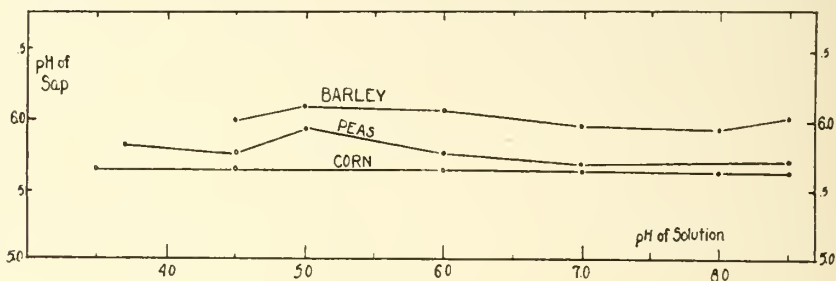


Fig. 6

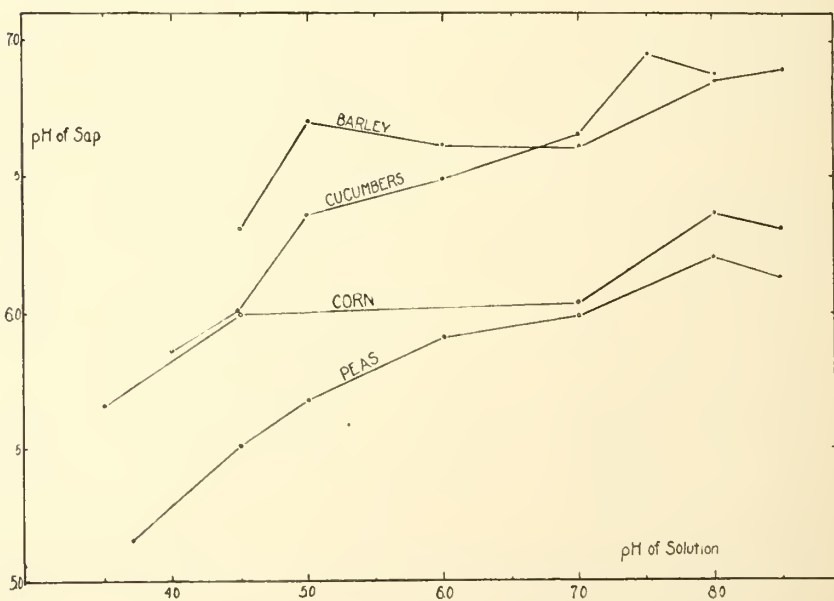


Fig. 7

of the plant, the ability to overcome any unfavorable reaction is correctly expressed by these curves. From a purely theoretical point of view, however, this ability may be determined at different reactions for plants treated similarly up to the time of experimentation, so that the vigor and internal mechanism of all the plants will as nearly

as possible be the same when subjected to the different acidities or alkalinities. These must be such that the plant mechanism will not be injured or altered materially during the period of experimentation.

Five sets of 25 barley plants each were grown in earthenware crocks of $7\frac{1}{2}$ liter capacity. All the solutions had a reaction of P_H 6.8, and were changed weekly. When plants were four weeks advanced, the sets were transferred to solutions having the reactions 4.0, 5.0, 6.0, 7.0, and 8.0, and these were kept as constant as possible for four days by the addition of N/5 acid or alkali. It was assumed that the sets of plants were not affected materially by the differences in reactions within this period of time.

In figure 5, the amounts of N/5 acid or alkali neutralized are plotted against the desired P_H as before. Unfortunately the number of determinations made are insufficient to permit of the smoothing out of the curves. Their general shape, however, is obvious. On the alkaline side of P_H 6.8, the ability to neutralize excessive concentrations of hydroxyl ions increases very rapidly with the increase in P_H and probably does not reach a maximum even at P_H 8.0. On the acid side, however, the increase is less rapid and reaches a maximum between P_H 4.0 and 5.0.

INFLUENCE OF FACTORS OTHER THAN THE REACTION

The plants were grown in the open during the summer months and in a heated greenhouse during winter. In the course of the investigation, it became evident that plants grown at different seasons show slight differences in their resistance to the effect of the reaction. This is most probably due to the differences in the rate of growth under different atmospheric conditions.

The influence of the composition of the culture solution on the effect of the reaction was not determined, as only one solution was used throughout the investigation. It is highly improbable, however, that the composition of the solution, within wide limits, is a factor in any of the divers phases of this study. The results obtained by Salter and McIlvaine²⁷ and those obtained by the writer seem to substantiate this assumption.

The amounts of water transpired by plants from solutions of different reactions were found to be the same within the limits of the experimental error.

DISCUSSION

The conclusion reached by earlier workers^{2, 4, 22} was that the H ion was more toxic than the OH ion to plants growing in solution cultures. Their results, however, are untenable because they failed to distinguish between potential and actual acidity or alkalinity. The ability of the plant to change the reaction of the nutrient medium was likewise overlooked.

In a series of papers, Hoagland^{15, 16, 17, 18} has called attention to both these factors and showed that the OH ion is much more toxic to barley seedlings in solution culture than the H ion. An OH ion concentration greater than P_H 8.2 was distinctly injurious, whereas an H ion concentration of P_H 5.0 was found to be favorable to growth and to cause no injury. Similar results were obtained by Duggar⁵ using various types of solutions and growing the plants under the most diverse environmental conditions. One of the most complete and satisfactory studies on this problem is that of Salter and McIlvaine.²⁷ These investigators experimented with corn, wheat, soybeans, and alfalfa, growing the plants at seven different H ion concentrations. The plants were grown for relatively short periods of time and the solution changed once every four days. A distinct maximum in the growth of the plants was found at P_H 5– P_H 6. At a neutral reaction, decided decreases in the yields could be demonstrated.

We have already called attention to the advisability of growing the plants for a considerable length of time so as to overcome the influence of the food supply stored in the seed. Only in this way is it possible to obtain a true measure of the effect of the reaction of the solution. The growth periods employed by these investigators were undoubtedly too short. On the other hand, the variations in reaction caused by young plants are relatively small, so that plants grown in accordance with the technique they employed will give more reliable results if the experiment is discontinued after two weeks than if the plants are grown for a longer period of time. Our results agree substantially with those of these investigators.

Hixon¹⁴ found a distinct minimum in the development of young plants as measured by the growth in length of the roots and tops at P_H 5 for *Pisum* and P_H 6 for most other plants. This minimum point is interpreted as that of greatest efficiency and the point of normal growth. We have been able to confirm his results in part. A decided stimulation occurred at acidities which injured the plants definitely

later on. No stimulation was noticed in the tops of plants grown in alkaline solutions. The roots were occasionally longer than those of the plants grown at P_H 5 or P_H 6.

A glance at figures 4 and 5 is sufficient to make evident the importance of controlling the reaction of the solution under investigation.

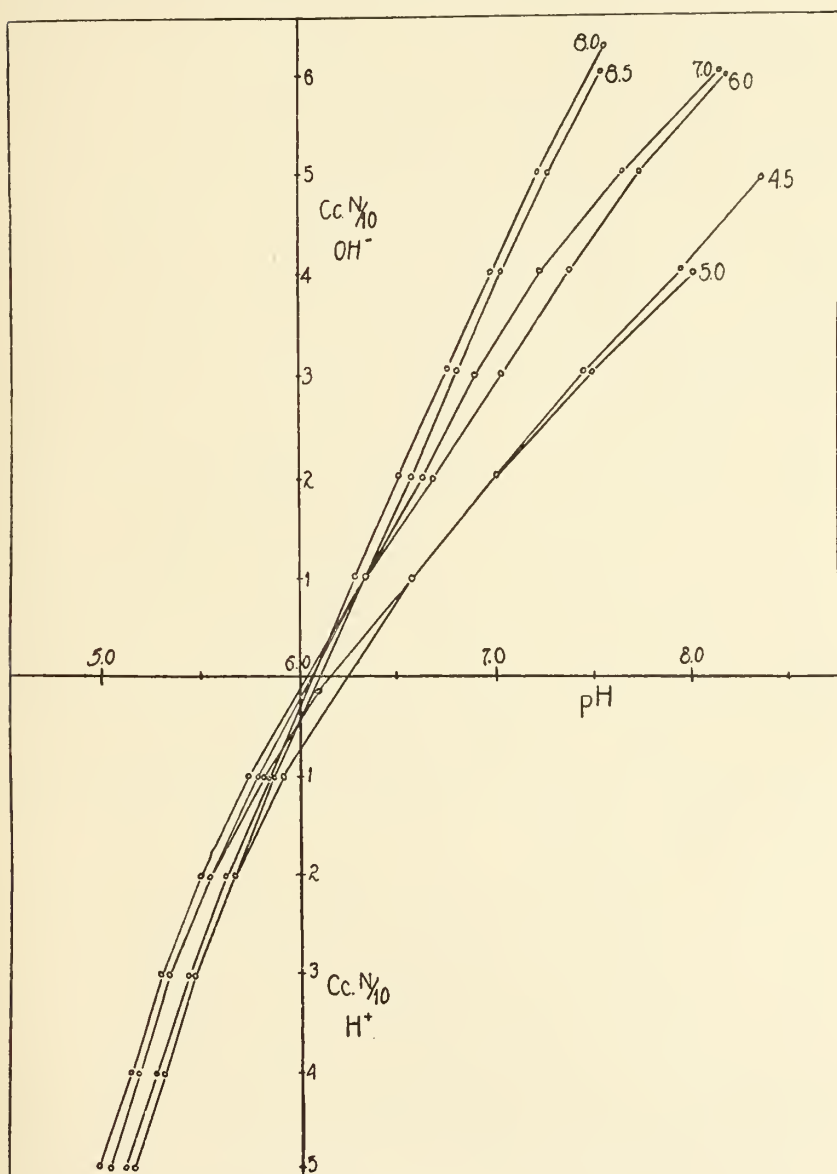


Fig. 8

Changes of solution every fourth or fifth day are obviously insufficient to maintain the P_H constant even approximately, when the plants are three to four weeks advanced. With small volumes of solutions supporting relatively large numbers of plants grown at P_H 5, this becomes increasingly difficult. In an investigation by McCall and Haag,²³ this point seems to be lost sight of completely. From their investigations, it appears that wheat plants grow best at reactions between P_H 3 and P_H 4. It is very plain, however, that the reactions of the solutions in the neighborhood of the roots must have been very different from what they were assumed to be. It is not strange that the solution with the highest buffer effect gave the poorest growth.

In culture solutions, the diffusion of solutes is relatively rapid and as a rule the reaction around the roots is the same as that in the bulk of the solution. If, however, the free diffusion is interfered with, such as often happens among the roots in the upper few inches of the solution, the reaction may be very different in this region from what it is in the bulk of the solution. Over the ranges of low buffer effect, a difference of 0.5 P_H can occasionally be demonstrated under such conditions. In soils, the diffusion is infinitely slower and the reaction of the solution in contact with the absorbing roots will be determined solely by the ability of the plant to overcome the buffer effect of the soil complex in its immediate vicinity. Considering the power of growing plants to regulate the P_H value of the culture medium, the conclusion is inevitable that the direct effect of the actual reaction of most soils can hardly be a factor in the complex which determines the growth of the plant in that soil, provided the plant has the ability to establish itself firmly. In this connection the work of Joffe¹⁹ with alfalfa is very elucidating. The results obtained with solution cultures agree well with those of this investigator using soils acidified artificially.

From the determination of the reactions of numerous acid soils reported by Gillespie,⁹ and Sharp and Hoagland,²⁸ it is apparent that the reaction of the majority of these soils can have little or no direct effect on the growth of plants. The infertility of acid soils can usually be ascribed to causes other than the H ion concentration. The solubility of aluminum in the slightly acid soil solutions of these soils is undoubtedly responsible for some of the phenomena attributed formerly to the acidity of the soil.^{1, 12, 24, 25, 29}

The soil solution of many alkali soils has a highly alkaline reaction, which tends to prevent the young plants from germinating or develop-

ing. Germinating seeds have a remarkable ability to change the reaction of the alkaline medium in which they are immersed, in the direction of neutrality, so that the P_H value around the seeds may be made favorable to germination. The ability of the seedling to

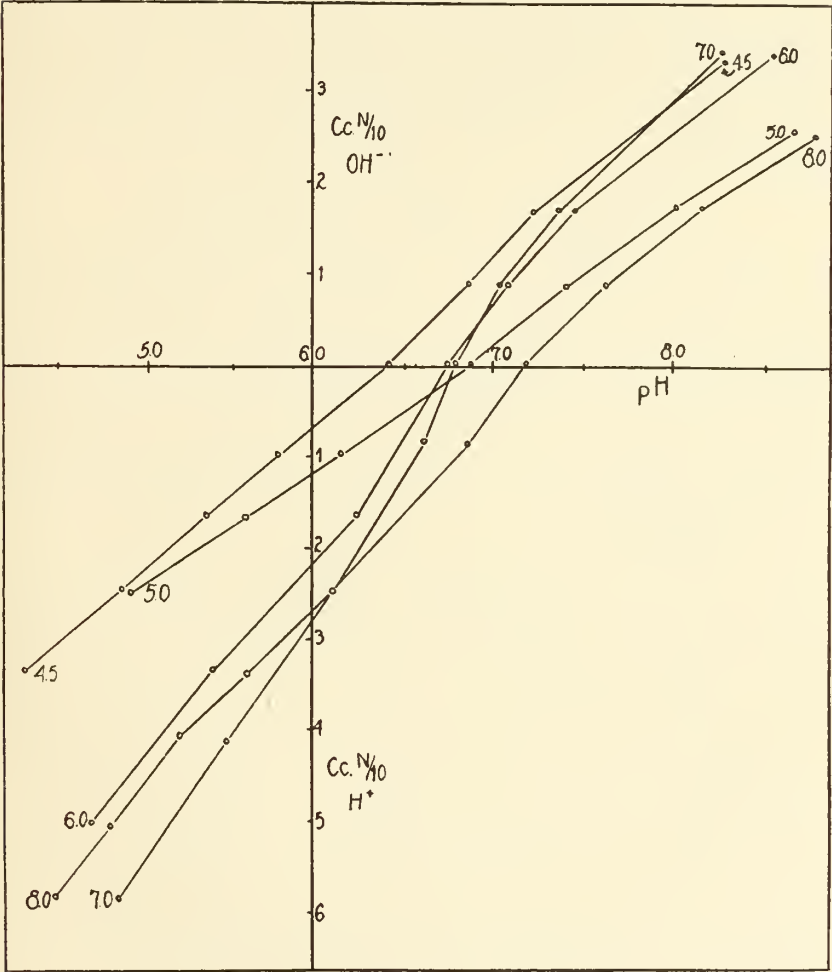


Fig. 9

regulate the reaction is comparatively small and hence the young roots may be unable to penetrate beyond the regions of the favorable reaction brought about by the seed. If the soil solution has both a high P_H value and a high concentration of salts, the seedlings will naturally be unable to survive.

EFFECT OF REACTION OF CULTURE SOLUTION ON THE REACTION AND
BUFFER EFFECT OF THE PLANT JUICES

The plants from the experiments described above were frozen immediately after they were harvested. This was done in a cold room kept at 12° F., from which they were only removed as they were needed. The plant juices were obtained by grinding the frozen mass, thawing this rapidly in a warm room, and then expressing the sap by hand through a few thicknesses of cheesecloth. All determinations were made as soon as possible after the frozen ground material was thawed out.

THE H-ION CONCENTRATION OF THE SAP

The reaction of the juices of the roots and tops, obtained in the above way, was measured by means of a Hildebrand hydrogen electrode. Difficulty was experienced in making the measurements as reduction of NO_3 ions apparently took place on the electrode. This was especially true in the case of the juices from those plants grown at the acid reactions. This difficulty was obviated to some extent by leaving the NaNO_3 out of the culture solution during the last week of the experiments.

In figures 6 and 7, the reactions of the tops and roots of cucumber, barley, pea, and corn plants grown at different reactions are represented graphically. The reactions of the sap expressed from the tops were not influenced by the reaction of the culture solution, the variations in reaction being within the limits of the experimental error.

On the other hand, the reactions of the root juices are decidedly changed by the reaction of the solution.* It is plain, however, that the reactions of the roots are very different from the reactions of the solutions, except when these are between P_H 6 and P_H 7. Whether the reaction of the root juices is influenced according to any definite rule by the reaction of the solution, as may be suggested by the curve for pea roots, it is impossible to say at present, owing to the relatively large experimental error involved in these measurements. Truog and Meacham,³¹ after studying the effect of additions of lime to a soil, concluded that the reaction of the soil can influence the reaction of the sap expressed from the tops of the plants. It seems obvious, however, that the differences in the reactions from the limed and unlimed plots

* Compare Bryan, O. C., Effect of different reactions on the growth and nodule formation of Soy beans. *Soil Science*, vol. 12, no. 4, pp. 271-287 (1922).

are within the experimental error, apart from the fact that many other factors enter in the case of plants growing in limed and unlimed acid soils.

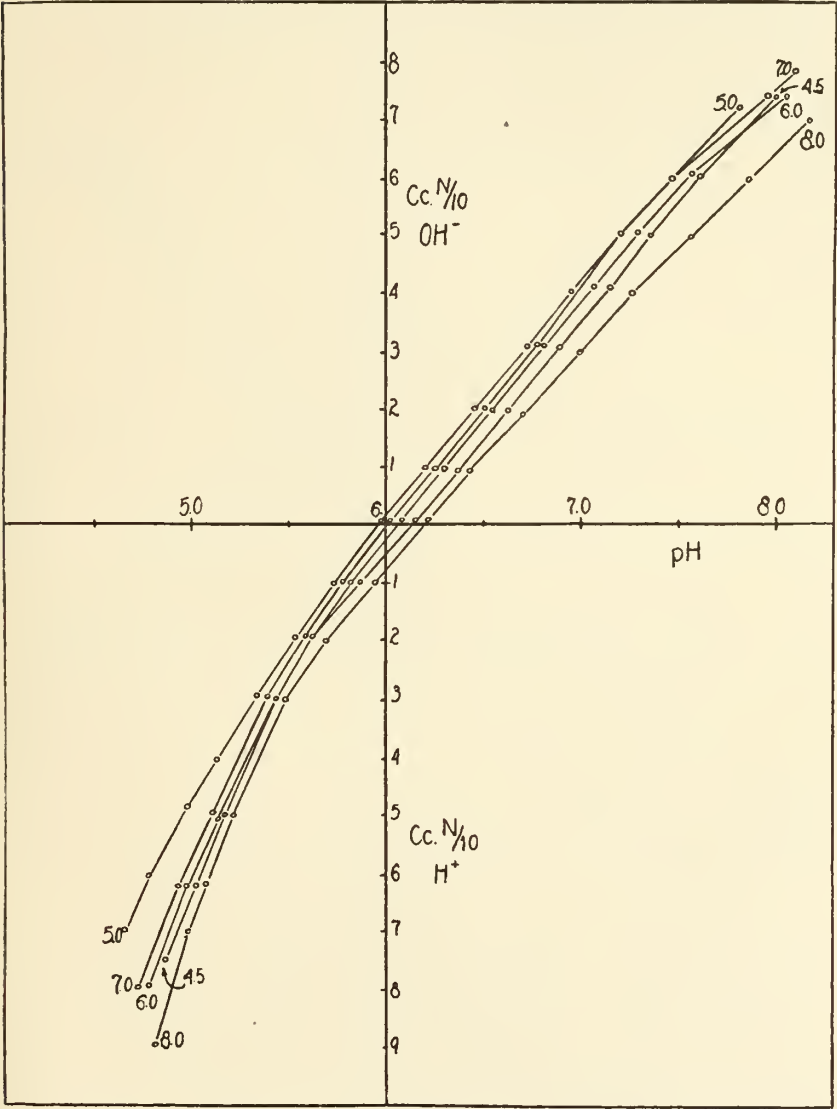


Fig. 10

THE BUFFER EFFECT OF THE SAP

The juice obtained from the plants in the way described was titrated electrometrically (after an equal volume of water had been added) with N/20 acid and alkali. The P_H value was invariably increased by about one-tenth of a magnitude of the dilution.

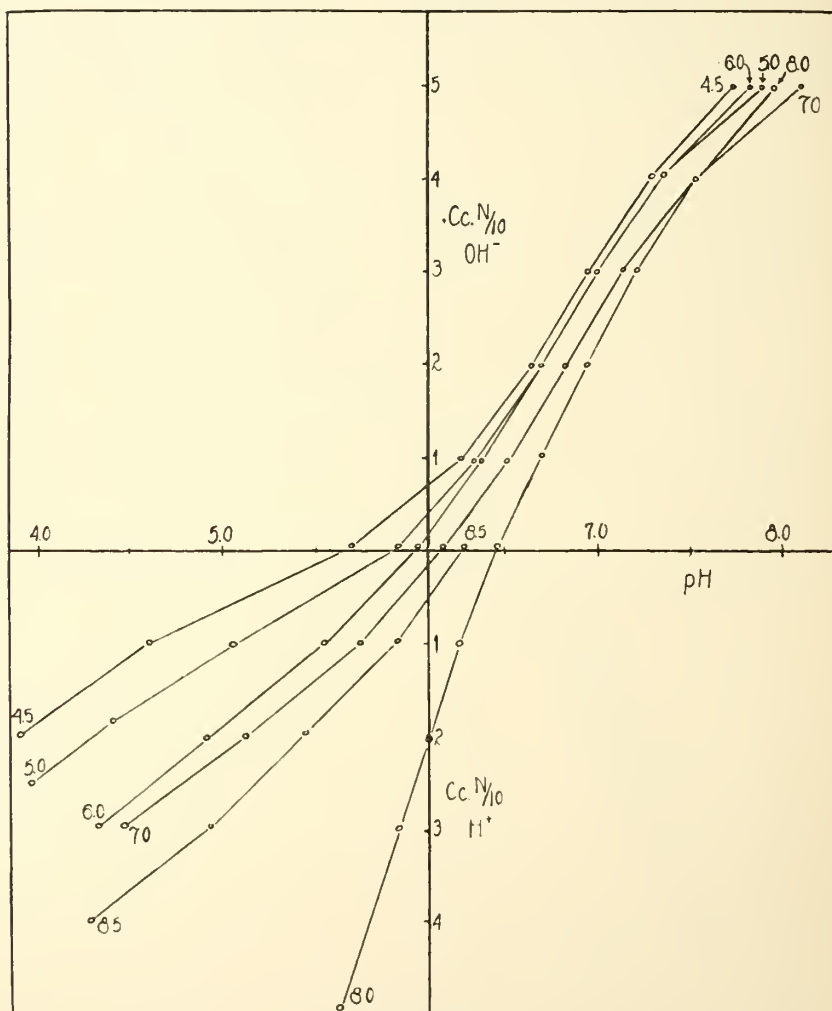


Fig. 11

In figures 8 and 9 the titration curves for barley tops and roots, respectively, are given. The curves are represented as if 25 c.c. of undiluted sap had been titrated with N/10 reagents. The corresponding curves for peas are given in figures 10 and 11.

Hempel¹³ has shown that the buffer effect of plant juices is mainly due to the organic acids and salts of these acids contained in the plant system. It appears from figure 8 that the reaction of the nutrient solution has influenced the concentration of those acids with dissociation constants less than 10^{-6} very markedly in the tops of barley plants although the reaction of the expressed sap is apparently unchanged.

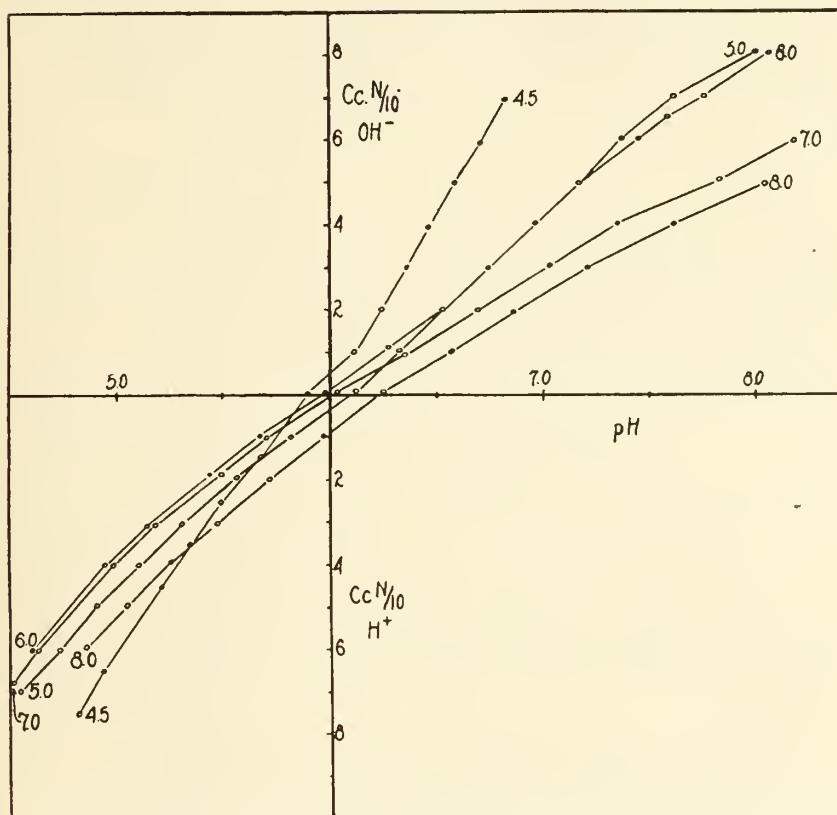


Fig. 12

In the roots the buffer effect is also influenced. Here, however, only those acids with a dissociation constant higher than 10^{-6} are affected. In the case of the pea plants, neither the reaction nor the buffer effect of the sap expressed from the tops was influenced by the reaction of the nutrient medium. The roots on the other hand were affected similarly to the barley roots. These plants were grown in a greenhouse during the winter.

In figure 12, the results of a similar experiment with peas grown in the open in summer are given. In this experiment, the reaction of

the sap expressed from the tops was unchanged, but the buffer effect was influenced by the reaction of the culture solution. The effect, however, is the reverse of what it was in the case of the tops of the barley plants, and in both instances was only noticeable in the concentration of those acids with a dissociation constant lower than 10^{-6} .

Unfortunately, it was not possible to pursue this line of investigation with additional plants and under the different atmospheric conditions. It seems, however, that a thorough study along these lines will throw considerable light on the salt metabolism of plants.

SUMMARY

1. The influence of the reaction of the culture medium on the growth and metabolism of the common agricultural plants was studied by growing typical plants in solution cultures at different reactions.

2. After experimenting with several different methods, a technique was devised by which the reaction of the solution could be conveniently controlled. Particular attention was given to the constant maintenance of the desired hydrogen-ion concentration during the experimental periods.

3. Plants grown in solution cultures have an optimum growth reaction at P_H 4.5 to P_H 6.

4. The reaction of the juice expressed from the tops of the plants was not influenced by the reaction of the culture medium, whereas the reaction of the juices expressed from the roots was modified considerably.

5. The buffer effect of both the roots and the tops may be influenced by the reaction of the culture solution. In the tops, the acid reserve is affected and in the roots, the alkali reserve.

6. Observations were made on the ability of the growing plant to change the reaction of either acid or alkaline culture solutions.

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II. POSSIBLE MECHANISM OF THE PLANT'S INFLUENCE ON THE REACTION OF THE CULTURE SOLUTION

Recent studies on the absorption of inorganic ions by plants as well as the large amount of work done on the problem of the antagonism between ions and the physiological balance in culture solutions have thrown some light on the mechanism by which the plant obtains its inorganic elements. The importance of a more thorough knowledge of this process is undisputed. Unfortunately investigations on this problem are hampered by our meager knowledge of the true nature of solutions and the methods of analysis at our disposal.

In considering the absorption of any ion, account must be taken of the activities of that ion inside and outside of the membrane effective in absorption. We are at present unable to determine the activity of any ion in a system as complex as a complete culture solution except that of the H ion, which can usually be determined with sufficient accuracy.

Since the activity and the total molal concentration of the H ion are conveniently and rapidly determined and since we have every reason to believe that the H and OH ions are absorbed, fundamentally, in the same way as any other positive or negative ion, we have here a very efficient means of studying this problem.

In Part I of this investigation, a series of experiments were described which were concerned mainly with the effect of the reaction of a culture solution on the growth and metabolism of several types of plants. In the present paper, some preliminary experiments are described which are concerned with the effect of the growing plant on the composition and especially on the reaction of culture solutions.

Pantanelli³ observed that plants always changed the reaction of a single salt solution in the direction of neutrality except when $(\text{NH}_4)_2\text{SO}_4$ was the solute. In a solution of this salt, the reaction remained at the initial value, namely P_{H} 5. Similar results were obtained by Hoagland⁴ with barley plants. Later work shows that solutions of $(\text{NH}_4)\text{Cl}$, K_2SO_4 and some other salts behave similarly to $(\text{NH}_4)_2\text{SO}_4$. The reaction may even change appreciably toward a higher acidity especially when the plants have not been previously grown in a complete culture solution. When complete culture solutions were used, the reaction was invariably changed toward neutral-

ity. These results with complete culture solutions were confirmed by Duggar² and several later workers for different types of plants and solutions. Jones and Shive⁵ found that the reactions of 20 representative solutions of the Tottingham series were changed toward neutrality. When, however, $(\text{NH}_4)_2\text{SO}_4$ was substituted for KNO_3 in these solutions, the reactions remained practically constant at the original value, namely P_H 4.8.

In the present investigation, all the plants experimented with invariably changed the reaction of the complete culture solution toward some point between P_H 6.5 and P_H 6.9, irrespective of what the original concentration might have been (see Part I).

The exact mechanism by which the plant changes the reaction of a solution has not been established. In a uni-salt solution, this may be ascribed to ionic exchanges, and on the alkaline side, the OH ions are partly neutralized by carbon dioxide excreted from the roots. In a complete culture solution, the problem becomes more complex. It will thus be of advantage to tabulate the different methods which a growing plant conceivably might have at its disposal for changing the reaction of the solution.

The decrease of H ion concentration would be accomplished:

1. By neutralizing H ions by OH ions derived from some base excreted by the roots or from dead root cells.
2. By absorbing H ions and simultaneously replacing these by some other positive ion.
3. By absorbing an anion and excreting OH ions simultaneously.
4. By absorbing H ions and an equivalent amount of some negative ion.
5. By absorbing an anion and excreting simultaneously another anion which forms an acid with a lower degree of dissociation or an acid which is volatile under the conditions.

The H ion concentration is increased:

- (a) If OH ions are neutralized by H ions derived from some acid excreted by the plant or from dead root cells.
- (b) If OH ions are absorbed but simultaneously replaced by some other anion.
- (c) If OH ions and an equivalent amount of a cation are absorbed simultaneously.
- (d) If a positive ion is absorbed and replaced by H ions.

Excretions by the roots are confined to the acid HCO_3^- (or CO_2) and, under certain conditions, small amount of cations, notably

calcium. The quantities of the latter are, however, insufficient to account for more than a very small part of the power of the plant to change the reaction of the solution. The increase in the P_H value must thus be accounted for by methods 3, 4, or 5.

The decrease in the P_H value of a culture solution might take place by any or all of the methods outlined under *a*, *b*, *c*, and *d*. In some cases the P_H value of the solution is decreased to about P_H 3.2, as frequently happens in a uni-salt solution of K_2SO_4 , for example. Since the concentration of the OH ions is very small at this reaction, it is possible that method *d* is chiefly involved. Method *c*, however, cannot be excluded from consideration.

The plant has a very efficient means at its disposal for reducing the alkalinity of a solution in that it normally excretes relatively large amounts of CO_2 (method *a*). This, however, is not the only mechanism involved as is apparent from the results of the following experiment. Corn plants growing in a complete culture solution maintained at P_H 8.5, as was described in Part I, neutralized within one week 0.0257 equivalents of alkali. When the solution was analyzed only 0.0185 equivalents of CO_2 were found. Hence approximately one-fifth of the alkali added must have been neutralized by methods *c* and *d*. Whether this neutralization was brought about with either or both of these methods, the final composition of the solution would be the same. The solution must have lost approximately 0.007 equivalents of cations, except H -ion and an equivalent amount of OH ions.

The conclusion, then, is inevitable that, exclusive of the H and OH ions, greater equivalent proportions of anions than of cations must be absorbed on the acid side. On the alkaline side, the reverse must be true.

The rate of absorption of either the anions or the cations, or both, may be influenced by differences in the reaction of the culture medium in order to bring about this selective absorption.

Several experiments were carried out to obtain some preliminary information on this point. The problem was attacked by means of absorption studies, the relative amounts of the different ions absorbed at different reactions by similar plants being determined. For the purpose of these experiments, actively growing four-week-old plants were used. The plants were grown in earthenware crocks, the solution used being identical with the culture solution described in Part I, except that KNO_3 was substituted for $NaNO_3$. The reactions were maintained at certain definite values by means of the technique

described in Part I, and the absorption was allowed to take place over a period of from 3 to 4 days, after which the solutions were made up to the original volume and analyzed.

The results of experiments with barley and cucumber plants are given in tables 1 and 2, respectively. Similar experiments were carried out with peas.

TABLE 1

ABSORPTION OF ANIONS AND CATIONS BY BARLEY PLANTS AT DIFFERENT REACTIONS

Period of absorption	No. of plants	pH at which solution was maintained	Total weights absorbed, gms.				Mg
			NO ₃	PO ₄	K	Ca	
3 days	30	4.5	.7144	.1824	.222	— .018	.023
3 days	30	8.0	.5952	.1856	.293	.006	.038

TABLE 2

ABSORPTION OF ANIONS AND CATIONS BY CUCUMBER PLANTS AT DIFFERENT REACTIONS

Period of absorption	No. of plants	pH at which solution was maintained	Total weights absorbed, gms.			
			NO ₃	PO ₄	K	Ca
3 days	25	5.0	.2557	.0646	.060	— .0010
3 days	25	6.0	.2425	.0420	.105	.0101
3 days	25	7.0	.2020	.0720	.180	.0169

It is apparent that the influence of the reaction is most marked on the rate of the absorption of the cations. Invariably there were more cations absorbed from the alkaline than from the acid solutions, i.e., the rate of absorption of cations was increased by a decrease in the concentration or activity of the H ion and vice versa. This implies a relative increase of the activity of the cations in the solutions over that in the plant. It is more probable that the activity of the cations in the plant is decreased than that the activity of the cations in the solution is increased so as to bring about such a marked change in the rate of absorption of the cations.

Loeb's⁶ brilliant researches have thrown much light on the relation existing between inorganic salts or ions and charged organic colloids and the distribution of ions on the two sides of a membrane when one side contains an ion which cannot diffuse through the membrane. To what extent the principles discovered by him may apply to the absorption of salts by plants it is impossible to say at present. If it be assumed that ionic equilibria are established between the roots

and the solution, these principles will undoubtedly determine the equilibrium concentration of the ions. Unfortunately, however, the existence of such equilibria in plant cells has not been established definitely. Assuming, however, that an equilibrium is established between the ions in the cells, which are active in absorption, and the ions in the solution, the effect of the reaction on the rate of absorption of the cations is readily explained as will be apparent from the following considerations.*

In the cell there are, among other substances, anions of organic acids, salts of these acids and probably of free acids, to which the membranes effective in the absorption are impermeable, and also complex colloidal bodies which are probably negatively charged (see below). Since the reaction of the cells of the roots (in so far as it is reflected in the plant juices) is influenced markedly by the reaction of the solution (see Part I), it is clear that an acid reaction of the culture solution will have the effect of depressing the dissociation of these acids and negatively charged bodies, and consequently the number of cations held by electrostatic forces will be decreased, i.e., the activity of the cations will be increased. In other words, the rate of absorption of the cations will be decreased by an increase in the H ion concentration. As a rule, a greater number of equivalents of nitrate ions were absorbed on the acid than on the alkaline side, whereas the absorption of phosphate ions was very irregular. Unfortunately, the ease with which a plant is able to replace certain anions absorbed by HCO_3 ions complicates attempts to determine whether or not the rate of absorption of anions is affected by the reaction of the culture solution. If the root contains any positively charged bodies to which the membranes are not permeable, we would expect an increase in the H ion concentration to produce an increased rate of absorption of the anions.

The charge on the proteins and other amphoteric bodies which constitute protoplasm becomes of paramount importance in this connection. To gain some information on this point resort was made to cataphoresis experiments. A slightly modified form of the apparatus described by Cohn, Gross, and Johnson¹ was used for the purpose,

* Since this paper was completed, work has been reported by the Laboratory of Plant Physiology of Harvard University and by the Laboratory of Plant Nutrition of the University of California, which indicates that additional considerations must be taken into account. For example, experiments on the alga *Nitella* (from which uncontaminated cell sap may be obtained) prove that an ion may be absorbed from a solution of low concentration into a solution of high concentration, and that certain inorganic elements, such as potassium, exist in the cell almost entirely in ionic form.

and the migration of the nitrogenous constituents in the sap, expressed from the roots after freezing, was determined by analyzing the buffer mixtures in the cathodic and anodic chambers for nitrogen by the Kjeldahl method. In the case of the juices from the roots of barley, pea, and cucumber plants, the migration was invariably found to be toward the anode, proving that these bodies are charged negatively at the reaction at which they occur in the plant. The direction of migration was not reversed at a reaction of P_H 4.5. The same results were found when the root juices were well dialysed against distilled water. Since the reaction of the juice, when it is freshly expressed from the tissue, has a reaction of approximately P_H 6, it is plain that the isoelectric points of the nitrogenous bodies are considerably below the P_H values at which they normally occur in the sap.

Deductions drawn from experiments with the expressed sap can hardly be considered as applying to the living root, which is a highly differentiated structure. The process of freezing may bring about changes sufficiently severe to change the sign of the charge on some of the ampholytes in the living cell or to cause mutual precipitation of oppositely charged colloids from the same or from different cells. In general it is improbable, however, that the isoelectric points of the different ampholytes will be changed materially by this treatment. Since the former are so far removed from the reaction at which these ampholytes occur in the root tissues, it is highly probable that the majority of the proteins and other nitrogenous bodies are charged negatively in the living cell, and that the sign of the charge is not readily reversible as assumed by Haynes.³ The work of Meier⁷ substantiates the above conclusions. This investigator found that the cell contents in the roots of actively growing plants moved under the influence of a small current as if they were negatively charged.

If a plant be allowed to change the reaction of an acid or alkaline solution, a certain P_H must be reached at which the tendency of methods 3, 4, and 5 to decrease the H ion concentration is balanced by the tendency of methods *a*, *c*, and *d* to decrease the OH ion concentration. Because of the many factors involved in this equilibrium, one can hardly expect this reaction to be very definite under the varying conditions of experimentation. For barley and corn, this value was found to be P_H 6.75 to P_H 6.8, and for peas, P_H 6.65 to P_H 6.7.

The significance of this point is not known at present. The main factor involved in bringing about this reaction in a solution seems to

be the activities of the CO_2 , H_2CO_3 , and the ions of this acid in the plant and in the solution. If this equilibrium is disturbed in such a way as to allow the escape of CO_2 , as may happen when the volume of the solution is diminished excessively by transpiration, the P_H value must rise. This is easily demonstrated by allowing the solutions in the containers to 'run down.' The reaction may rise to as high as P_H 8.5. On the other hand, if the other factors which contribute toward this equilibrium be missing, the P_H value will decrease till an equilibrium is established between the CO_2 of the atmosphere above the solution and the H_2CO_3 and HCO_3^- in the solution. Such a condition is brought about in distilled water in which the reaction is maintained at a slight acidity. The equilibrium reaction also depends upon the rapidity with which the different ions are absorbed. In solutions in which the rapidly absorbed anion NO_3^- is replaced by the rapidly absorbed cation NH_4^+ , as in the investigation by Jones and Shive,⁵ the equilibrium will naturally be thrown over to the acid side.

From the above considerations, it is obvious that an 'optimum' culture solution for the growth of plants will depend not only on the composition of the culture solution, but also upon the partial pressure of the CO_2 in the atmosphere and other atmospheric conditions.

The fact that the optimum reaction for the growth of plants in solution culture is on the acid side is possibly correlated in part with the greater ease with which the respiratory CO_2 can diffuse out and away from the roots at this reaction. If this theory is correct, the optimum reaction will even be slightly more toward the acid side in soil, since the diffusion of CO_2 is interfered with.

SUMMARY

1. A study of the effect of the reaction of the solution on the absorption of the anions and cations by the plant is described.
2. Several methods are outlined by which the plant changes the reaction of either acid or alkaline culture solutions toward neutrality.
3. Absorption experiments show that the rate of absorption of the cations is increased by a decrease in the H-ion concentration, while the ability of the plant to excrete CO_2 from the roots allows of the selective absorption of anions from the acid solutions.
4. The charge on the constituents of the root cells may be assumed to be of vital importance in the mechanism of absorption.

5. The nitrogenous constituents of the cell sap are charged negatively and the isoelectric points of the majority of ampholytes in the cell is below P_H 4.5.

6. Pea plants change the reaction of either acid or alkaline solutions from P_H 6.65 to P_H 6.7, whereas barley and corn plants change it from P_H 6.75 to P_H 6.8. The main factor involved in bringing about this reaction is the CO_2 - HCO_3^- equilibrium in the plant and in the solution.

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